

Fig. S1. Biophysical characterization of the Sgt2-C_{cons} domain. (A) CD spectra as in Fig. 1C for the conserved C-terminal domains of ySgt2 (blue) and hSgt2 (orange). NMR spectra as in Fig. 1D & E for ySgt2-C_{cons} (B, blue) and hSgt2-C_{cons} (C, orange).

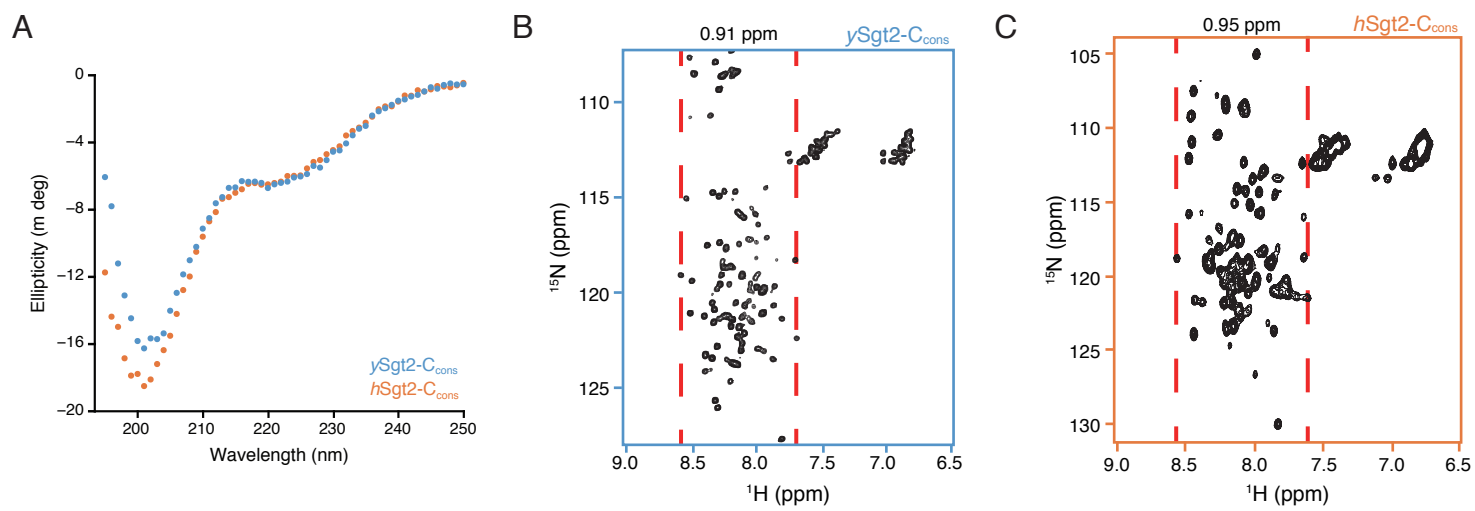


Figure S1

Fig. S2. Identification of minimal binding region of Sgt2. (A) The full image of the gel in Fig 2C. (B) An anti-MBP western blot of the lysate from the which the complexes in (A) were purified from. The load concentrations were normalized based on the total optical density of the cells when harvested.

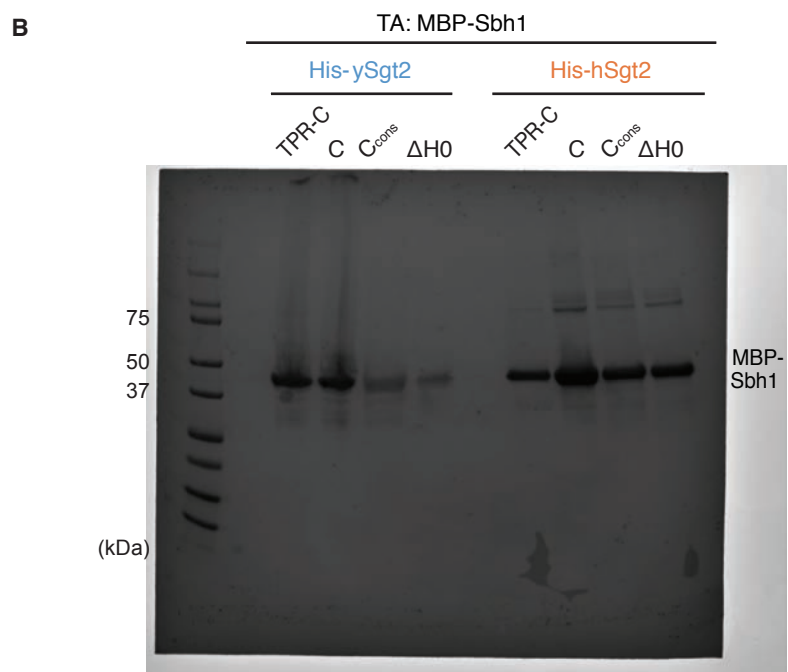
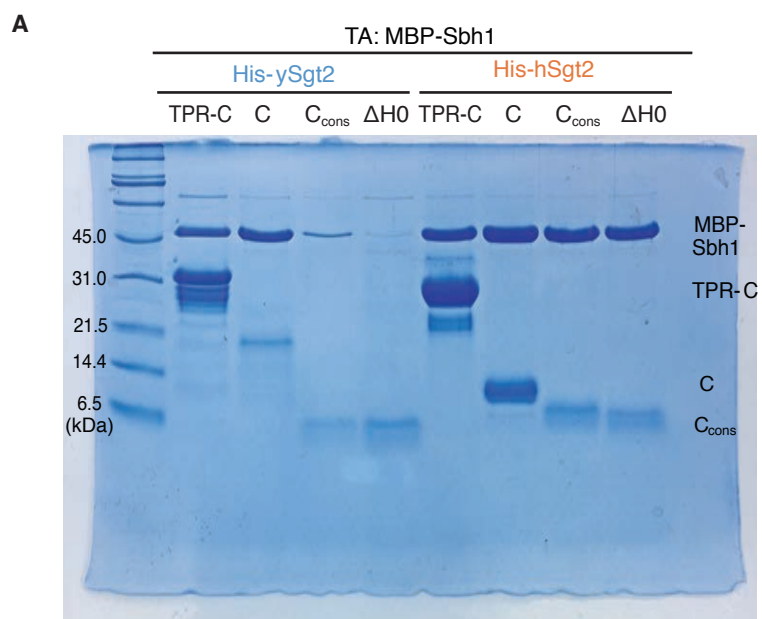


Figure S2

Fig. S3. Structural models across prediction methods. (A) Predictions from Quark, I-TASSER, Pcons, Phyre2, RaptorX, and Robetta. Methods produce between 5 and 10 models. (B) Robetta provides a residue-wise estimated error in Angstroms; this is shown below the corresponding models with a grey bar indicating the C_{cons} region.

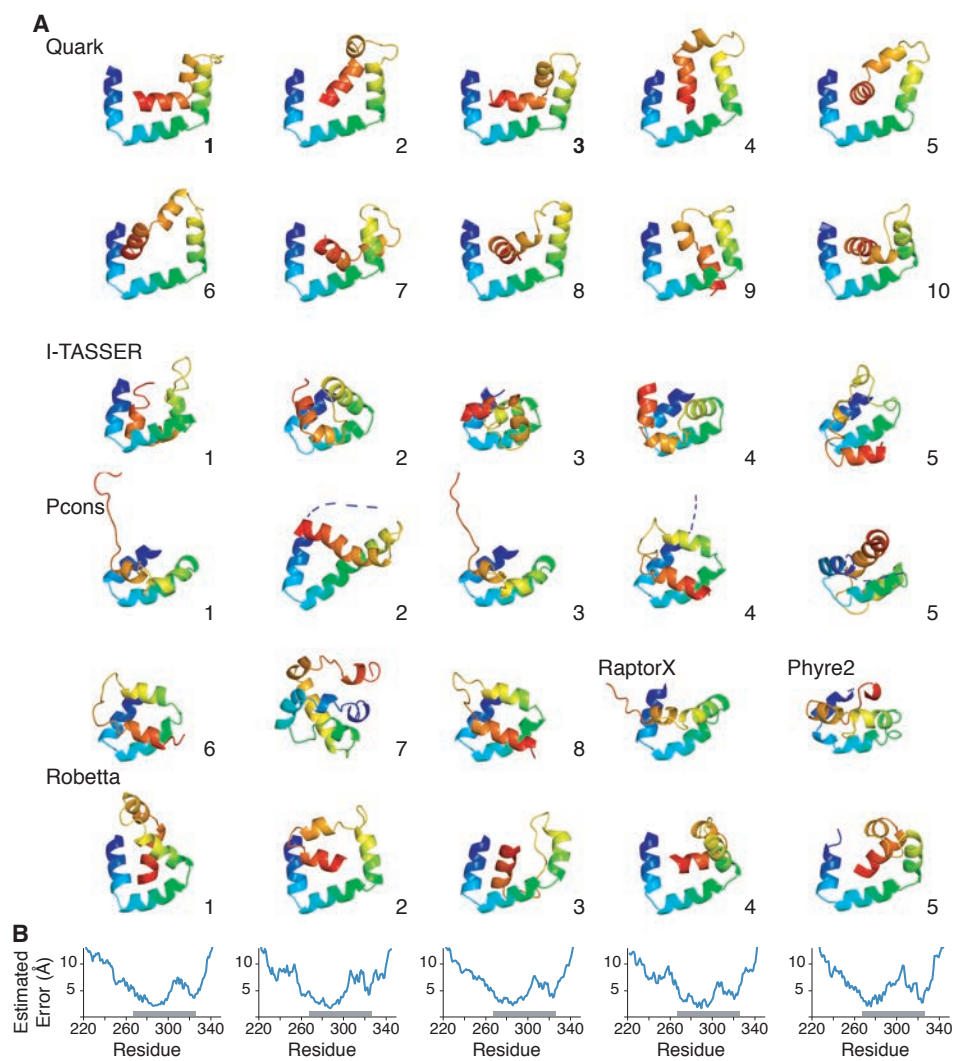


Figure S3

Fig. S4. Cysteine mutants are capable of binding to clients. (A) Schematic showing how his-tagged ySgt2-TPR-C and double cysteine mutant constructs were coexpressed with the client 11[L8], and complexes were purified by nickel affinity chromatography. (B) A coomassie stained SDS-PAGE gel of the elution fractions demonstrates that 11[L8] was present in the elution suggesting double cysteine mutations do not affect client binding. (C) An anti-cMyc western blot of the fractions represented in the SDS-PAGE gel also demonstrates that 11[L8] was present in all eluates.

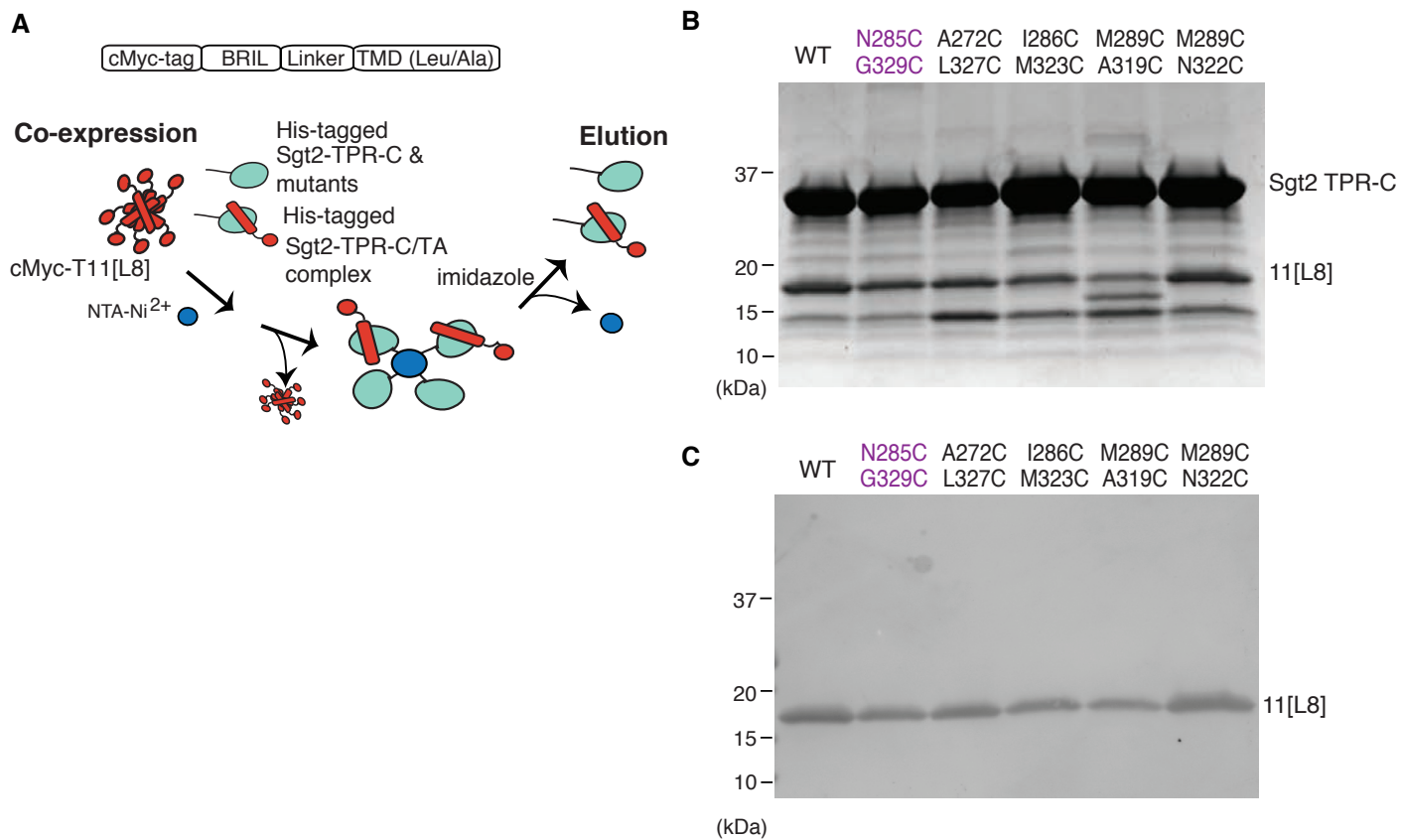
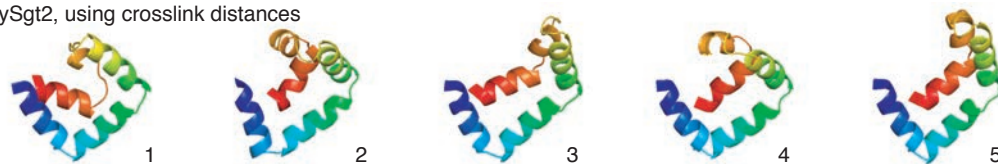


Figure S4

Fig. S5. Distance restraints lead to improved ySgt2-C and suggestive hSgt2-C models. (A)

Prediction of ySgt2-C using distances from *in vitro* crosslinking. (B) C $_{\beta}$ -C $_{\beta}$ distances between residues probed by *in vitro* disulfide crosslinking for each ySgt2 model. Distances 9 Å or less are colored orange. For models where all distances correspond (4 near and 1 far), the row is shaded grey. (C) Models for hSgt2-C using restraints, adding a N-terminal loop, and via the new Robetta TR method.

A ySgt2, using crosslink distances



B

	N285-G329	A272-L327	I286-M323	M289-A319	M289-N322	
1	18.6	4.6	6.8	7.3	12.4	
2	10.3	5.6	5.5	9.0	7.4	
3	12.7	6.7	4.8	5.0	8.0	
4	9.6	8.2	7.7	9.0	6.7	
5	8.5	8.0	7.2	7.2	5.1	

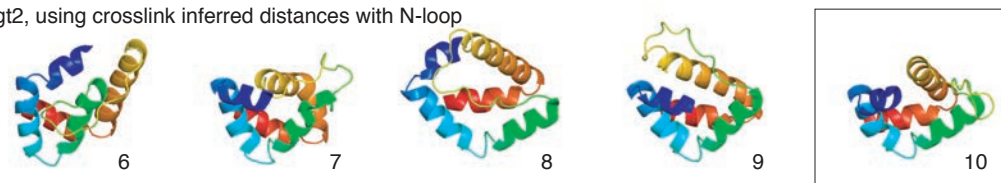
C_β-C_β
Distance (Å)
9 Å or less

C

hSgt2, using crosslink inferred distances



hSgt2, using crosslink inferred distances with N-loop



hSgt2, Robetta TR

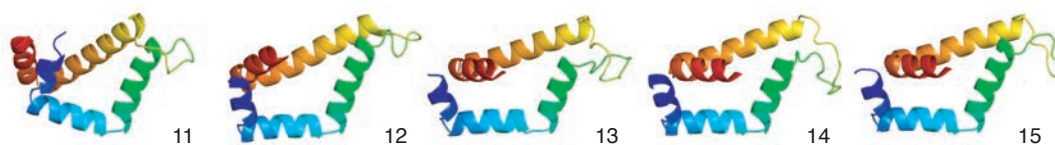


Figure S5